Analytical electron microscopy of calcium carbonates and calcium phosphates in crustacean calcium bodies

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Calcium bodies are specialised bacteria-harbouring organs that accumulate calcium minerals in terrestrial crustaceans. They contain mineralized extracellular matrices that are cyclically deposited and resorbed during the animals' moult cycle and likely function as reservoirs for calcium salts, which constitute an important component of the crustacean exoskeleton. Surprisingly, the calcified matrix of calcium bodies in most species that possess them is filled with numerous bacteria, the function of which is currently unknown. In one remarkable species, the woodlouse *Hyloniscus riparius*, two different pairs of these organs are present. The posterior pair is filled with bacteria whereas the anterior pair lacks bacteria. Bacteria-containing calcium bodies continuously accumulate calcium phosphate. The bacteria-free calcium bodies, on the other hand, accumulate only calcium carbonate. This species is particularly interesting, as it may shed light on the function of bacteria in calcium bodies.

In the present study we analysed the mineral deposits in the two pairs of calcium bodies (Figure 1a) at high spatial and high energy resolution using analytical scanning transmission electron microscopy (STEM), which enabled us to determine the fine-scale structure and composition of the mineral deposits in calcium bodies. Isolated calcium bodies were dehydrated in methanol and embedded in resin. In order to preserve highly soluble amorphous calcium carbonate, dry ultrathin sections were prepared. The mineralised matrices were imaged and analysed with the Zeiss SESAM and JEOL ARM200F microscopes. Energy-dispersive X-ray spectroscopy (EDX) and electron energy-loss spectroscopy (EELS) were used to characterise the Ca-containing minerals. Selected area electron diffraction (SAED) experiments were performed with a Zeiss 912 Omega transmission electron microscope.

As demonstrated with EDX and SAED, the bacteria-filled matrix of the posterior calcium bodies is continuously mineralized with calcium phosphate in the form of apatite crystals a few hundred nanometres in size (Figure 1b). The transient glassy layer that is deposited between the bacteria and calcium body epithelium during preparation for moult is mineralised with a mixture of amorphous calcium carbonate and amorphous calcium phosphate. The mineralised matrix in the anterior calcium bodies of *H. riparius*, which lacks bacteria, consists entirely of calcium carbonate with no demonstrable amounts of phosphorus. As demonstrated with SAED and EELS, the matrix in anterior calcium bodies is mineralised with amorphous calcium carbonate.
As the glassy matrix layer of the posterior calcium bodies and the mineralised matrix of the anterior calcium bodies are transient and are completely resorbed after moult, their mineralisation with amorphous calcium minerals is likely biologically functional due to their high solubility under physiological conditions. Our results indicate that the presence of bacteria may be essential for the turnover of calcium phosphate in calcium bodies. Further studies of this unique relationship between bacteria and animals in a biomineralisation process may shed light on the role of bacteria in pathological mineralisation processes in humans.

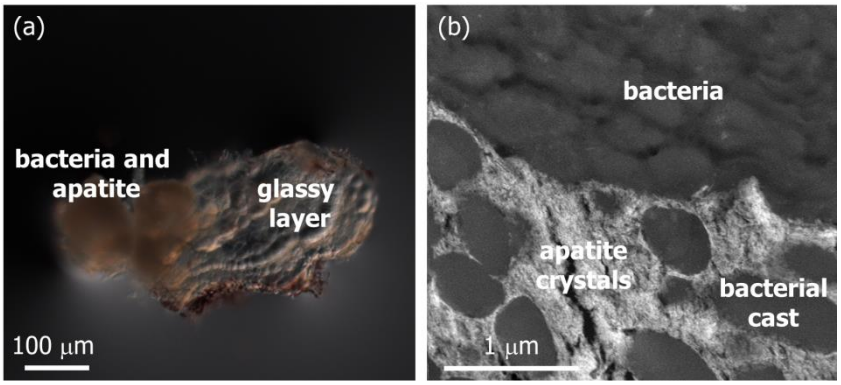

Figure 1: (a) Light micrograph of the posterior calcium body matrix. (b) HAADF-STEM image of the apatite crystals and bacteria in a posterior calcium body.